

We claim:

1. A method of analyzing the protein content of a population of cells from a tissue sample, comprising:
 - a) extracting the population of cells from the tissue sample using laser capture microdissection;
 - b) isolating a protein sample from the extracted cell population; and
 - c) analyzing the isolated protein sample.
2. The method of claim 1 wherein isolating the protein sample comprises solubilizing the extracted cell contents in a small volume of a buffer comprising at least one detergent to solubilize the cellular lipids, at least one proteinase inhibitor to preserve protein content and function, and at least one salt to lyse the nuclear contents.
3. The method of claim 2 wherein the small volume of buffer is about 1 μ l to about 15 μ l.
4. The method of claim 1 wherein analyzing the protein sample comprises performing a soluble immunoassay using a labeled antibody specific for a protein of interest.
5. The method of claim 4 wherein the labeled antibody is labeled with a marker selected from the group consisting of colorimetric-detectable, chemiluminescence, fluorescence, and radioactivity.
6. A method of quantifying the amount of a protein of interest in a population of cells, comprising:
 - a) extracting the population of cells from the tissue sample using laser capture microdissection;
 - b) isolating a protein sample from the extracted cell population by solubilizing the extracted cell contents in about 1 μ l to about 15 μ l of a buffer where the buffer comprises Tris-HCl, NP-40, sodium deoxycholate, sodium chloride, EDTA, aprotinin, leupeptin, sodium pyrophosphate, sodium orthovanadate, and AEBSF; and
 - c) performing a soluble immunoassay using an antibody specific for a protein of interest in the protein sample, where the antibody is labeled with a marker selected from

the group consisting of colorimetric-detectable, chemiluminescence, fluorescence, and radioactivity wherein the assay is calibrated to indicate the amount of the protein of interest present in the cell population.

7. The method of claim 6 wherein the protein of interest is prostate soluble antigen (PSA).

8. The method of claim 1 wherein analyzing the isolated protein sample comprises:
a) performing a one dimensional polyacrylamide gel electrophoresis (1D PAGE) or two dimensional polyacrylamide gel electrophoresis (2D PAGE) to separate proteins from each other; and
b) further analyzing the proteins using a protein specific dye or Western blotting with a labeled antibody specific for the protein of interest.

9. The method of claim 1 wherein analyzing the protein sample comprises
a) performing a two dimensional polyacrylimide gel electrophoresis (2D PAGE) to separate the proteins from each other;
b) isolating a protein of interest from the gel; and
c) determining an amino acid sequence of the protein of interest.

10. The method of claim 9 wherein the sequence is determined using a method selected from the group consisting of N-terminal sequencing, mass spectrometry MS-MS sequencing, liquid chromatography quadrupole ion trap electrospray (LCQ-MS), and matrix assisted laser desorption/time of flight analysis (MALDI/TOF).

11. The method of claim 1 wherein analyzing the protein sample comprises performing surface enhanced laser desorption ionization spectroscopy (SELDI) to produce a protein fingerprint for the cell population.

12. The method of claim 1 wherein the cell population is microscopically identifiable as a tumor cell.

13. A method of characterizing binding properties of one or more intracellular proteins of a population of cells, comprising:

- 5 a) extracting the population of cells from the tissue sample using laser capture microdissection;
- b) performing a one dimensional polyacrylimide gel electrophoresis (1D PAGE) or two dimensional polyacrylimide gel electrophoresis (2D PAGE) to separate the proteins from each other;
- 10 c) removing at least one protein of interest from the gel;
- d) further analyzing the protein of interest by incubating the protein with a known or putative binding partner for the protein of interest; and
- e) determining whether the protein of interest binds to the known or putative binding partner.

15 14. The method of claim 13 wherein the protein of interest is PSA and the known binding partner is alpha-1-antichymotrypsin (ACT).

15. A method of differentiating a protein content of several populations of cells of a tissue sample, comprising the steps of:

- 20 a) extracting at least a first and a second population of cellular material directly from one or more tissue samples using laser capture microdissection;
- b) isolating protein from the extracted cell populations;
- c) analyzing the isolated protein; and
- d) comparing a protein content of the first cell population to a protein content of the second cell population to identify the differing content.

25 16. The method of claim 15 wherein isolating protein comprises solubilizing the extracted cellular material in a small volume of a buffer wherein the buffer comprises Tris-HCl, NP-40, sodium deoxycholate, sodium chloride, EDTA, aprotinin, leupeptin, sodium pyrophosphate, sodium orthovanadate, and AEBSF.

17. The method of claim 15 wherein the small volume of buffer is about 1 μ l to about 15 μ l.
18. The method of claim 15 wherein analyzing the isolated protein comprises performing a soluble immunoassay using a labeled antibody specific for a protein of interest wherein the assay is calibrated to indicate the amount of the protein of interest present in the cell population.
19. The method of claim 15 wherein the immunoassay is of high sensitivity and the labeled antibody is labeled with a marker selected from the group consisting of colorimetric-detectable, chemiluminescence, fluorescence, and radioactive labels.
20. The method of claim 15 wherein analyzing the isolated protein comprises:
- a) performing a two dimensional polyacrylamide gel electrophoresis (2D PAGE) to separate proteins from each other;
 - b) isolating a protein of interest from the gel; and
 - c) determining an amino acid sequence of the protein of interest.
21. The method of claim 20 wherein the sequence is determined using a method selected from the group consisting of N-terminal sequencing, mass spectrometry MS-MS sequencing, liquid chromatography quadrupole ion trap electrospray (LCQ-MS), and matrix assisted laser desorption/time of flight analysis (MALDI/TOF).
22. The method of claim 15 wherein analyzing the isolated protein comprises:
- a) performing a one dimensional polyacrylamide gel electrophoresis (1D PAGE) or two dimensional polyacrylamide gel electrophoresis (2D PAGE) to separate protein fractions from each other; and
 - b) further analyzing the protein fractions using a protein specific dye or Western blotting with a labeled antibody specific for a protein of interest.
23. The method of claim 15 wherein the first population of cells and the second population of cells are from the same tissue sample and the first population is microscopically identifiable as tumor cells and the second population is microscopically identifiable as normal cells.

24. The method of claim 15 wherein the first population comprises several subpopulations wherein each subpopulation is microscopically identifiable as cells at different stages of tumor progression.

25. A method of comparing the protein content of a first population of cells
5 microscopically identifiable as tumor cells to the protein content of a second population of cells that are normal wherein both populations of cells are extracted from the same tissue sample, the method comprising:

a) extracting the first and second populations of cells from the tissue sample using laser capture microdissection, in which a laser targets the first and second populations as
10 microscopically distinct and separates them from a larger microscopic structure;

b) isolating a protein sample from each extracted cell population by solubilizing the extracted cell contents in about 1 μ l to about 15 μ l of a buffer where the buffer comprises Tris-HCl, NP-40, sodium deoxycholate, sodium chloride, EDTA, aprotinin, leupeptin, sodium pyrophosphate, sodium orthovanadate, and AEBSF; and

15 c) performing a one dimensional polyacrylamide gel electrophoresis (1D PAGE) or two dimensional polyacrylamide gel electrophoresis (2D PAGE) to separate proteins of the protein sample from each cell population;

d) further analyzing the separated proteins of each cell population using a protein specific dye or Western blotting with a labeled antibody specific for a protein of interest; and

20 e) comparing a protein of interest content of the first cell population to a protein of interest content of the second cell population.

26. A method of comparing the protein content of a first population of cells microscopically identifiable as tumor cells to the protein content of a second population of cells in order to identify the origin of the first population of cells, the method comprising:

25 a) extracting the first and second populations of cells from the tissue sample and from each other using laser capture microdissection;

b) isolating a protein sample from each extracted cell population by solubilizing cells from extracted cell populations in about 1 μ l to about 15 μ l of a buffer where the buffer

comprises Tris-HCl, NP-40, sodium deoxycholate, sodium chloride, EDTA, aprotinin, leupeptin, sodium pyrophosphate, sodium orthovanadate, and AEBSF;

c) performing surface enhanced laser desorption ionization spectroscopy (SELDI) to produce a protein fingerprint of the protein sample for each cell population; and

5 d) comparing the protein fingerprint of the first population of cells to the protein fingerprint of a known second population of cells to determine whether or not the two populations have the same origin.

27. The method of claim 26 wherein said first population of cells is microscopically identifiable as a tumor metastasis and the second population of cells is a battery of known normal
10 tissue samples.

28. The method of claim 27 wherein the known normal tissue samples are from the same patient as the first population of cells.

29. A device for isolating protein from a population of cells collected by laser capture microdissection, comprising a chamber in which are present a population of cells obtained by laser
15 microdissection, at least one input port connected to the chamber with a canal having the ability to move liquid introduced into the inlet port by capillary action, a supply of liquid in communication with the inlet port for isolating a protein sample from the population of cells, and at least one output port.

30. The device of claim 29 wherein the chamber is structured to allow the direct
20 acceptance of a cap used to collect cells in the laser capture microdissection process.

31. The device of claim 29 wherein the at least one input port is provided with a means to introduce volumes of liquid into the inlet port and canal.

32. The device of claim 31 wherein said means is a syringe.

33. A device for isolating protein from a population of cells collected by laser capture
25 microdissection, comprising a chamber, three input ports having syringes for the introduction of small volumes of liquid into the chamber, where the input ports are connected to the chamber with canals having the ability to move liquid introduced into the inlet port.

34. A method of screening for the presence of a cellular component in a population of cells from a tissue sample, comprising:

a) extracting a population of cells from the tissue sample using laser capture microdissection;

5 b) lysing the extracted cell population to produce cellular components;

c) immobilizing at least one cellular component or a binding agent in a confined zone;

d) contacting the cellular components with the binding agent; and

e) detecting the interaction between the components and the binding agent.

10 35. The method of claim 34 wherein the cellular component or the binding agent is labeled, and detecting the interaction between the cellular component and the binding agent comprises detecting the presence of the label.

36. The method of claim 35 wherein the label is detected by a method selected from the group consisting of a colorimetric, chemiluminescent, radioactive, and fluorescent label.

15 37. The method of claim 34 wherein the confined zone of the immobilized cellular component or the immobilized binding agent is an array.

38. The method of claim 34 wherein the cellular component is immobilized.

39. The method of claim 34 wherein the binding agent is immobilized.

20 40. A method of screening for the presence of a cellular component in a population of cells obtained by laser capture microdissection from a tissue sample, comprising:

providing an array that includes either (a) an array of immobilized binding agents for the cellular component or (b) an array of immobilized cellular components from the microdissected cells;

25 exposing the array of immobilized binding agents to laser microdissected cellular components, or exposing the array of immobilized cellular components to binding agents for cellular components of interest.